Rapid and high-throughput screening of 3 cannabinoids in cell culture medium using the Echo® MS system from SCIEX

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ABSTRACT

Synthetic biology is an emerging field of biological research in the early 21st century. It involves the design and construction of novel artificial biological pathways, organisms and devices or the redesign of existing natural biological systems to develop compounds such as drugs, functional materials or energy substitutes. Due to the need to screen different strains and reaction conditions, a large number of samples are generated, and timely analysis is needed. In this work, the Echo® MS system from SCIEX was used for the rapid analysis of 3 cannabinoids in cell culture medium. The samples were extracted with ethyl acetate and diluted with acetonitrile/ water and submitted directly for analysis. This method has the advantages of simple pretreatment, small sample consumption and a very short analysis time, which is suitable for rapid and high-throughput screening of complex samples in synthetic biology research.

INTRODUCTION

The traditional way to obtain secondary metabolites is to extract them from plants or through chemical synthesis. However, chemical synthesis is costly, so it is an ineffective way to obtain cannabinoids in large quantities.[1, 2] Biosynthesis of cannabinoids by microbial strains has become an effective means of obtaining cost-effective, high-quality cannabinoids. Synthetic biology can be used to generate chassis organisms to study and characterize enzymes associated with the biosynthesis of these lower levels of cannabinoids, and their derivatives, and to produce these compounds through scalable fermentation processes. In addition, by incorporating custom enzymes, microbial production can yield novel cannabinoids with enhanced properties.[3, 4]

Based on the plant cannabinoid biosynthesis pathway, the industrial production of cannabinoids or the synthesis of new cannabinoid drugs can be achieved using synthetic biology, and great progress has been made in this field. In the course of synthetic biology research, it may be necessary to develop and evaluate thousands of strains and select suitable conditions to optimize production, from which strains with excellent performance and optimal conditions can be obtained to scale up commercial production. Due to the large number of variable experimental factors, it is often necessary to conduct quantitative analysis of thousands of samples in the experimental process, so a suitable high-throughput data acquisition method is needed to quickly get the results.

The Echo MS system is a high-throughput sample analysis system based on Acoustic Ejection Mass Spectrometry (AEMS) combined with electrospray ionization. It can be used to analyze matrix samples by a relatively simple sample pretreatment method. Combined with the Open Port Interface (OPI) sampling technique, no carryover is typically seen during the sampling process. The nanoscale sample is introduced into the carrier solvent, which dilutes the sample prior to mass spectrometry.

Here, the Echo® MS system was used to rapidly analyze 3 cannabinoid components in cultured cells, including olivetolic acid (OA), cannabigerolic acid (CBGA) and cannabidiolic acid (CBDA). This method has the advantages of simple pretreatment, small sample consumption and short analysis time, which is suitable for rapid and high-throughput screening of complex samples in synthetic biology studies.

MATERIALS AND METHODS

Sample preparation: The stock solution of 3 cannabinoids was diluted by 1:1 (*v/v*) acetonitrile/water. The cell medium samples were centrifuged, extracted with ethyl acetate and evaporated to dryness under nitrogen. The samples were reconstituted in 100 µL of 1:1 (*v/v*) acetonitrile/water. The samples (60 µL) were added to each well of a 384-well plate.

Acoustic ejection method parameters: Methanol containing 2 mM ammonium fluoride was used as carrier solvent and the operational flow rate was 400 µL/min. A sample volume of 12.5 nL was ejected into the mass spectrometer for analysis.

MS method parameters: The Echo MS system includes a SCIEX Triple Quad 6500+ mass spectrometer and was controlled by SCIEX OS software 3.1. The 3 compounds were analyzed by multiple reaction monitoring (MRM) acquisition. The optimized MS parameters are listed in Table 1.

Data processing: Data processing was performed using SCIEX OS software. A calibration curve was generated for each compound with 6 replicates at each concentration level to evaluate ejection reproducibility and accurately determine the lower limit of quantitation (LLOQ). The calibration curves are shown in Figure 1 and a quantitation summary is listed in Table 2. The total ion chromatogram (TIC) is shown in Figure 2.

Table 1. Optimized MS parameters.

Name	Q1/Q3 (m/z)	DP (V)	CE (V)
Olivetolic acid	223.0/179.1	-60	-27
Cannabigerolic acid	359.2/341.2	-60	-29
Cannabidiolic acid	357.2/245.1	-60	-42
Source parameters	**Value**	**Source parameters**	**Value**
Curtain gas (psi)	20	CAD gas (psi)	8
Ion source gas 1 (psi)	90	Ion spray voltage (V)	5000
Ion source gas 2 (psi)	50	Source temperature (°C)	300

RESULTS

Eight different concentrations of standard samples analyzed in 6 replicates demonstrated the reproducibility of AEMS. Excellent CV (%) was achieved at all concentration levels. At the LLOQ, the CV for consecutive ejections was < 10%. As summarized in Table 2, the assay accuracies of 89-111% and CV (%) were well within standard acceptance criteria for all tested samples. The calibration curves were generated across 3 orders of linear dynamic range, with a correlation coefficient (*r*) > 0.99.

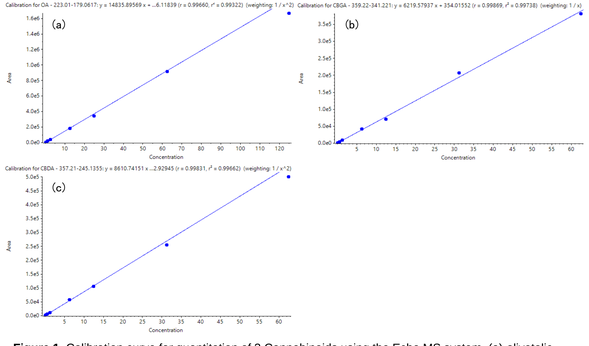

Figure 1. Calibration curve for quantitation of 3 Cannabinoids using the Echo MS system. (a) olivetolic acid; (b) cannabigerolic acid, (c) cannabidiolic acid.

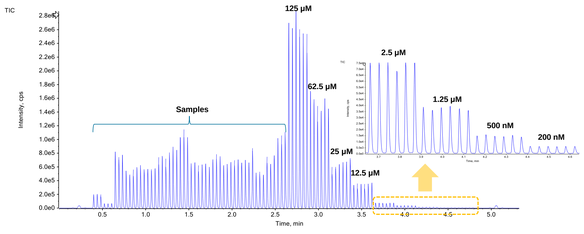

Figure 2. TIC of olivetolic acid.

Table 2. Quantitation summary for the analysis of 3 cannabinoids analysis using the Echo MS system

Name	Actual concentration (µM)	Calculated concentration (µM)	Accuracy (%)	CV (%)	N
Olivetolic acid	0.20	0.20	97.07	4.65	6
	0.50	0.51	101.41	5.00	6
	1.25	1.37	109.95	4.01	6
	2.50	2.77	110.68	1.89	6
	12.50	12.49	99.93	1.94	6
	25.00	23.10	92.38	5.97	6
	62.50	61.72	98.74	4.20	6
	125.00	112.29	89.83	4.78	6
Cannabigerolic acid	0.10	0.10	103.04	9.68	6
	0.25	0.23	91.11	7.59	6
	0.625	0.62	99.24	4.93	6
	1.25	1.32	105.95	3.66	6
	6.25	6.60	105.60	3.49	6
	12.5	11.32	90.59	6.84	6
	31.25	33.26	106.44	5.82	6
	62.50	61.26	98.02	8.61	6
Cannabidiolic acid	0.10	0.10	96.59	7.01	6
	0.25	0.27	106.58	2.01	6
	0.625	0.65	104.32	4.57	6
	1.25	1.26	100.38	7.01	6
	6.25	6.64	106.20	5.88	6
	12.5	12.27	98.17	4.90	6
	31.25	29.61	94.75	6.46	6
	62.50	58.13	93.01	5.82	6

CONCLUSIONS

The Echo MS system can meet high-throughput sample acquisition requirements and produce high-quality data, ensuring rapid and accurate quantitation of the 3 cannabinoid compounds in cell culture medium with simple sample preparation.

The very short analysis time (2 seconds per sample) enabled the rapid generation of quantitative data for high numbers of samples.

The assay showed great reproducibility without using labeled internal standards, although the use of labeled internal standards may further improve these results.

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